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Review

Prolyl isomerases in gene transcription☆

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ABSTRACT

Background: Peptidyl-prolyl isomerases (PPIases) are enzymes that assist in the folding of newly-synthesized proteins and regulate the stability, localization, and activity of mature proteins. They do so by catalyzing reversible (*cis-trans*) rotation about the peptide bond that precedes proline, inducing conformational changes in target proteins.
Scope of Review: This review will discuss how PPIases regulate gene transcription by controlling the activity of (1) DNA-binding transcription regulatory proteins, (2) RNA polymerase II, and (3) chromatin and histone modifying enzymes.
Major Conclusions: Members of each family of PPIase (cyclophilins, FKBPs, and parvulins) regulate gene transcription at multiple levels. In all but a few cases, the exact mechanisms remain elusive. Structure studies, development of specific inhibitors, and new methodologies for studying *cis*/*trans* isomerization *in vivo* represent some of the challenges in this new frontier that merges two important fields.
General Significance: Prolyl isomerases have been found to play key regulatory roles in all phases of the transcription process. Moreover, PPIases control upstream signaling pathways that regulate gene-specific transcription during development, hormone response and environmental stress. Although transcription is often rate-limiting in the production of enzymes and structural proteins, post-transcriptional modifications are also critical, and PPIases play key roles here as well (see other reviews in this issue). This article is part of a Special Issue entitled Proline-directed Foldases: Cell Signaling Catalysts and Drug Targets.

1. Introduction

PPIases were discovered in 1984 by Gunter Fischer (Editor of this special issue) and colleagues as enzymes that catalyze the *cis*/*trans* isomerization of peptide bonds that precede the amino acid proline (X-Pro) [1]. Rotation about these bonds within proteins is normally restricted due to its partial double-bonded character and steric hindrance between adjacent α-carbons. (Fig. 1A). The *cis-trans* interconversion does not require ATP, but instead uses energy derived from conformational changes in the protein substrates [2,3]. Prolyl isomerases typically accelerate the *cis-trans* isomerization of the peptide bond within peptide substrates by a factor of 10^3–10^6 [4–6]. Spontaneous and catalyzed isomerization rates on intact protein substrates are difficult to measure, but are assumed to be much lower than for peptides due to additional steric considerations. PPIases or "foldases" as they were originally called, help in the folding of nascent proteins, but also induce conformational changes in mature proteins, thereby regulating their activity and/or and interaction with other proteins [7,8].

There are three distinct families of PPIase, the cyclophilins (CyPs), the FKBPs (FK506 binding proteins), and the parvulins [9–12] (Fig. 1B). All three are conserved amongst eukaryotes, prokaryotes and archaea [12–15], with family size increasing with proteome complexity. For example, in the budding yeast, *Saccharomyces cerevisiae* there are 8 cyclophilins and 4 FKBPs, while in humans there are 18 cyclophilins and 16 FKBPs [11,16]. In yeast there is one parvulin, Ess1 (*ess*ential in yeast), while in humans there are two parvulins, Pin1 (human ortholog of Ess1; *p*rotein *i*nteracting with *N*IMA), and Par14/17 which are protein isoforms encoded by the same gene [17–21]. Each family of PPIase has structurally distinct catalytic domains and they exhibit some differences in substrate specificity [22–24]. In eukaryotic cells, PPIases can be found in all cellular compartments including the cytoplasm, endoplasmic reticulum, mitochondrion, nucleus and nucleolus [25].

The cyclophilins and FKBPs can be divided into two broad categories, the single domain and the multidomain PPIases. The single domain proteins are abundant and are composed of essentially only the isomerase catalytic domain (Fig. 2) and in the case of cyclophilins and FKBPs, these are thought to be the major targets of therapeutic drugs (discussed below). Multidomain cyclophilins and FKBPs contain additional distinct functional domains that mediate protein-protein or RNA-binding interactions. Most prominent among these is the TPR

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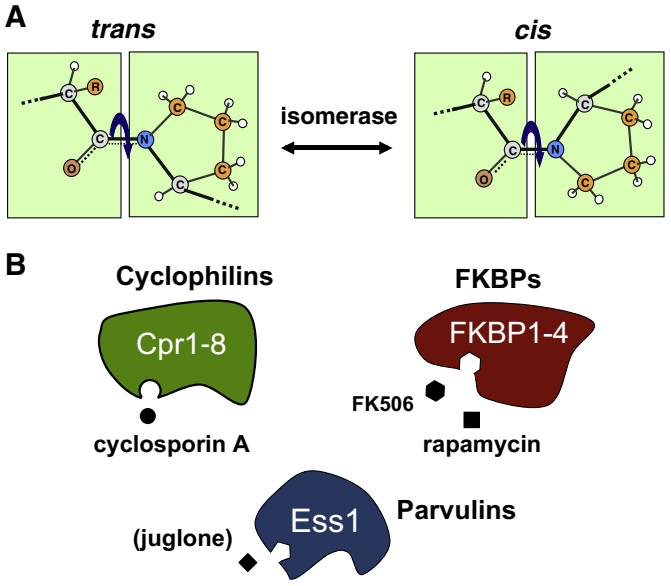

Fig. 1. (A) Depiction of the *trans* and *cis* isomeric forms of an X-peptide bond and the interconversion stimulated by prolyl isomerases. (B) Three major families of prolyl isomerase and their respective inhibitors. Juglone is shown in parentheses to indicate it is relatively non-specific. Cyclosporin A, FK506 and rapamycin and derivatives thereof are commonly used as immunosuppressive drugs. Cpr1-8, FKBP1-4 and Ess1 refer to the yeast proteins.

(tetratricopeptide repeat), a degenerate 34-residue motif often found in tandem [26] (Fig. 2). One important interaction mediated by the TPR motif is between the immunophilin PPIases and steroid receptor complexes, as will be described below. Parvulins, by contrast, are all small proteins (*parvulus*, Latin for very small). In bacteria, parvulins are comprised of a PPIase domain [27], while in eukaryotes they can also contain a N-terminal WW-domain [28] followed by a short linker and a C-terminal catalytic domain [18,19,29] (Fig. 2). Note that both the WW-domain and the PPIase domain of Ess1 and its human ortholog Pin1, bind the same target sequence: phosphorylated Ser/Thr-Pro. Among the PPIases, only parvulins bind in a phosphorylation-dependent manner, a feature that is critically important for their function and has facilitated their study [30,31]. The WW-domain binds with ~10-fold higher affinity than does the PPIase domain and is thought to target Ess1/Pin1 to substrates [32].

Members of the cyclophilin and FKBP families are commonly referred to as immunophilins because they mediate the effects of immunosuppressive drugs cyclosporin A (CsA), FK506 and rapamycin [33–35]. These drugs bind in the respective active sites of the immunophilins and block PPIase catalytic activity (Fig. 1B). Oddly enough, however, their immunosuppressive effects do not result from inhibition of PPIase activity, but instead from gain-of-function interactions by the immunophilin-drug complexes. Cyclophilin A-CsA and FKBP12-FK506 complexes inhibit the phosphatase activity of calcineurin, which would normally activate the transcription factor NF-AT to promote T-cell activation [36,37]. The FKBP12-rapamycin complex inhibits the mTOR (target of rapamycin) kinase blocking signaling in T-cells in response to cytokine stimulation [38–40]. In addition to their clinical importance, these immunophilin-drug complexes serve as cautionary examples for why researchers who study PPIases must consider whether the catalytic activity of the enzyme is, in fact, critical to the biological process being studied, and what exactly is learned by using mutational and drug-inhibition studies. This issue was thoughtfully discussed in a recent review on FKBP25 [41], and will be noted below.

In contrast to the cyclophilins and FKBPs, the parvulins do not mediate immunosuppressive effects and do not bind CsA, FK506, or rapamycin. The relatively non-specific inhibitor, juglone [42], inhibits the activity of parvulins Ess1 and Pin1, and has been used extensively in mammalian cell culture experiments. However, given its rather general mechanism of inhibition (covalent modification of active-site cysteines), caution must be used interpreting results of *in vivo* inhibition studies, since many different enzymes might also be targeted. [43]. For example, juglone is a potent inhibitor of RNA polymerase II by blocking formation of functional pre-initiation complexes [44]. More specific inhibitors of parvulins have been isolated and engineered, but are not widely available. These include peptidomimetics such as D-isomer and cyclic peptides, and conformationally-locked substrates [43,45–50].

PPIases of all three major families as well as an orphan PPIase, called Rrd1 in yeast, have all been implicated in regulation of gene transcription. Gene transcription is a complex, highly-regulated process that requires the action of DNA-binding proteins, RNA polymerases, RNA modifying enzymes, and enzymes that modify DNA and its associated chromatin. This review is organized into three major sections, how PPIases control (1) DNA-binding regulatory proteins, (2) RNA polymerase II function, and (3) chromatin structure and histone modification. Related activities in which PPIases have been implicated, such as RNA splicing and DNA repair, will be mentioned briefly at the end. It should be noted that a number of excellent reviews covering selected aspects discussed here have been published elsewhere [11,16,51–58]. Here, I will emphasize the important concepts, provide specific examples, point out future questions, and provide the reader with references for further inquiry. A synopsis of some of what is covered below is offered in Table 1.

2. Control of transcription regulatory proteins by PPIases

2.1. Localization of transcription factors

In eukaryotes, transcription of nuclear genes relies on DNA-binding regulatory proteins (transcription factors) that are synthesized in the cytoplasm. A key regulatory step is the transport of these proteins into or out of the nucleus, a process often regulated by signal transduction pathways in response to extracellular ligands. A number of examples have emerged that show that PPIases target transcription factors to regulate nuclear-cytoplasmic shuffling. For example, nuclear import and activation of steroid hormone receptors, which will be considered separately below, is facilitated by TPR-containing FKBPs and cyclophilin 40 (CyP40). In yeast, cyclophilin A promotes the nuclear export of Zpr1, a zinc-finger protein required for growth, although its function as a transcription factor is not firmly established [59].

In mammalian cells, the parvulin Pin1 is reported to regulate the nuclear localization of a number of signaling proteins required for transcription factor induction as well as the transcription factors themselves. For example, Pin1 promotes nuclear localization of cyclin D, [60], β-catenin [61] and NF-κB [62], while it prevents nuclear localization of NF-AT [63], FOXO4 [64] and CRTC2 [65]. In each case, Pin1 binding to phospho-Ser-Pro motifs [30,32] affects nuclear localization, however, the underlying mechanisms are different. Pin1 binds to pSer246-Pro247 in β-catenin reducing its interaction with APC (adenomatous polyposis coli protein) [61], which normally shuttles β-catenin out of the nucleus [66]. Whether Pin1 acts stoichiometrically to physically block β-catenin interaction with APC, or whether Pin1 catalytic activity induces conformational changes in β-catenin to block interaction with APC is not known. In the case of NF-AT, Pin1 binds via its WW domain to a pSer-Pro site normally dephosphorylated by calcineurin, thereby trapping NF-AT in the cytoplasm [63]. In the case of the stress-responsive forkhead transcription factor, FOXO4, Pin1 binding promotes de-ubiquitylation of FOX04, preventing nuclear entry (which requires monoubiquitination) [64]. A Pin1 catalytic mutant did not affect levels of expression of FOXO4 target genes, suggesting that regulation of FOXO4 by Pin1 requires isomerase activity. Most recently, Pin1 was shown to bind the nuclear localization sequence (NLS) of CRTC2 at pSer136-Pro137 [65], although the exact mechanism of action

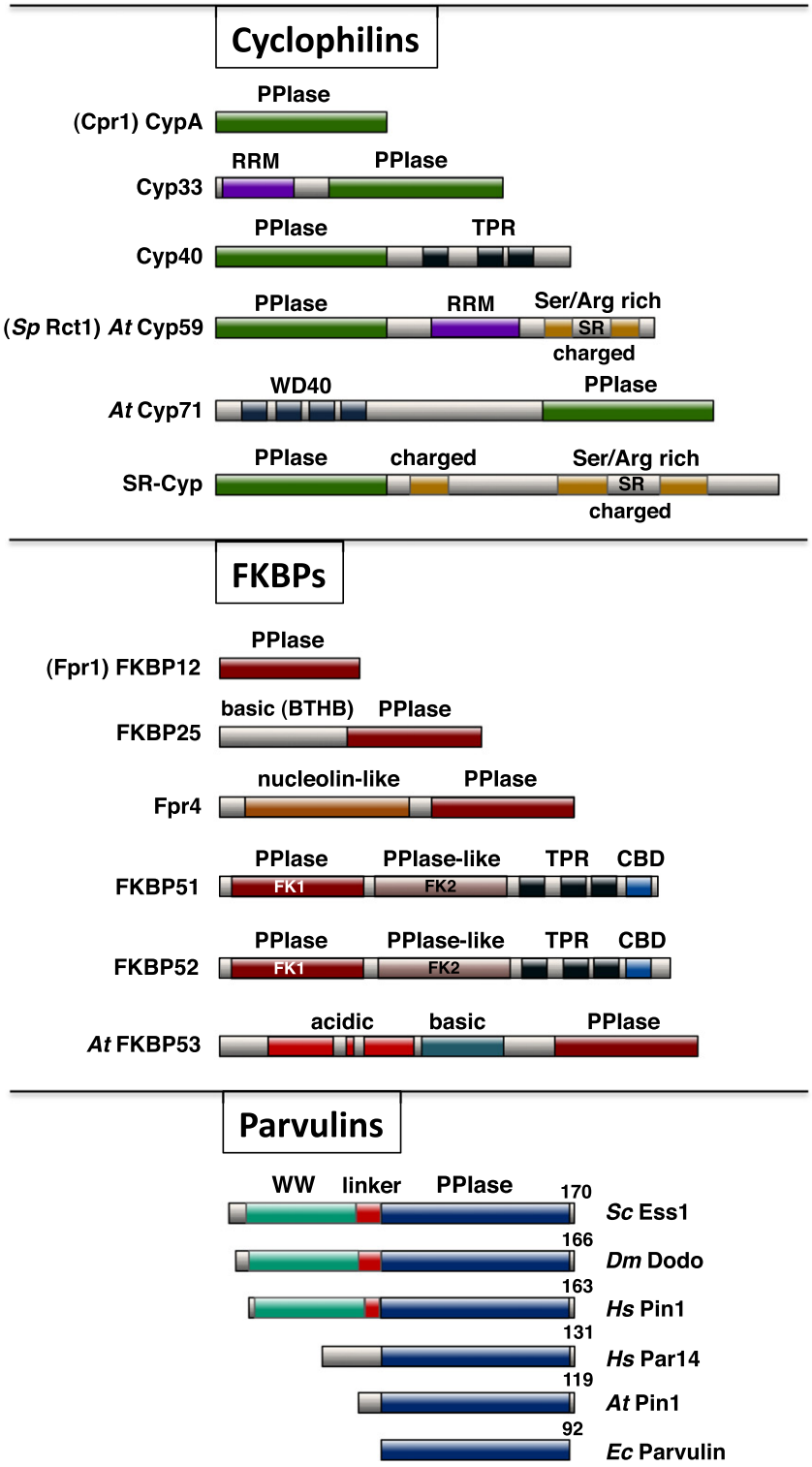

Fig. 2. Alignment and domain structure of PPIases discussed in this review, divided into major families. PPIase, catalytic domain; RRM, RNA-binding domain; TPR, tetratricopeptide repeat; FK1, active FKBP isomerase domain; FK2, inactive FKBP isomerase domain; CBD, calmodulin binding domain; WW, protein-interaction domain. This is not a complete set and is composed of PPIases from different organisms. If not indicated, the protein is from yeast or mammals. *Sp*, *S. pombe*; *At*, *A. thaliana*; *Sc*, *S. cerevisiae*; *Hs*, *human*; *Ec*, *Escherichia coli*.

by Pin1 and whether its catalytic activity is required is not known. These studies illustrate that parvulins influence the localization of proteins by both catalytic and non-catalytic means. The affects are likely to be indirect, via changes in substrate availability or conformation that in turn regulate covalent modification by kinases and/or phosphatases.

In another mechanism, Pin1 isomerization controls cleavage and relocation of Notch1 [67]. Initially, Pin1 and p53 were proposed to prevent processing of Notch from its transmembrane (inactive) form to its γ-secretase-processed, nuclear localized (activated) form [68]. A later study shows that Pin1 interacts directly with Notch1 to *stimulate*

Table 1
Summary of PPIases discussed in this review.

Name (Family)	Organism(s)[1]	Associated domain(s)[2]	Target(s)	Function(s)	Refs.
Cyclophlins					
Cpr1 (CypA)	*S. cerevisiae*	none	calcineurin Rpd3, Set3 (HDACs)	binds cyclosporin A causes cell cycle arrest; decreases silencing at rDNA; induction of meiosis-specific genes *IME1 IME2*; export of Zpr1	[11,36,59,153,212,213]
CypA	mammals	none	calcineurin	binds cyclosporin A, mediates immunosuppression by blocking T-cell activation	[33,37]
CypB	mammals	none	Prolactin receptor	Stimulates DNA binding and activation by Stat5, release of PIAS inhibitor	[98]
CsCyP	plants	none	RNA pol II-CTD	target of bacterial pathogen PthA2	[152]
Cyp33	mammals	RRM	RNA, MLL1, H3(?)	isomerizes MLL1, converts it to repressor, inhibits H3K4me3 and recruits HDACs; may isomerize H3 proline(s); may be regulated by RNA	[205–210]
Cyp40	mammals	TPR	c-Myb; steroid receptors	blocks c-Myb DNA binding; localization and activation of steroid receptors	[97,137–140]
At Cyp59	*Arabidopsis*	RRM, SR	SR-proteins, RNA pol II-CTD; RNA	Isomerase activity inhibited by binding specific 7 nt RNA sequence	[149,233]
Rct1	*S. pombe*	RRM, SR	SR-proteins, RNA pol II-CTD	CTD phosphorylation; RNA pol II elongation (?)	[150,151]
At Cyp71	*Arabidopsis*	WD40	LHP1, FAS1, H3	promotes H3K27 methylation, represses *HOX* genes, histone chaperone activity	[215,216]
SR-Cyp (Matrin)	mammals	SR, charged	RNA pol II-CTD	RNA splicing (?)	[146–148]
FKBPs					
Fpr1 (FKBP12)	*S. cerevisiae*	none	calcineurin, Tor kinase	binds FK506, rapamycin, drug-complexes cause cell cycle arrest	[38]
FKBP12	mammals	none	calcineurin, mTor, YY1	binds FK506, rapamycin, mediates immunosuppression by blocking T-cell activation; inhibits mTor signaling, stimulates activation by YY1	[34,35,39,40,99]
Fpr4	*S. cerevisiae*	nucleolin-like	histones H3, H4	rDNA silencing (NTS), histone chaperone; isomerizes H3-P16, P30, P38; keeps Set2-dependent H3K36me3 levels low until gene induction	[218,219,224]
FKBP25 (Fpr4 ortholog)	mammals	BTHB	YY1, nucleolin, MDM2	stimulates YY1 DNA binding, recruits HDAC1,2; ribosome biogenesis; degradation of MDM2/protects p53 from destruction	[100,101,229,93]
FKPB51/52	mammals	FK2, TPR, CBD	steroid receptors - AR, GR, MR, PR, ER; NF-κB;	FKBP51 acts negatively, FKBP52 acts positively as co-chaperones for steroid receptor activation, nuclear localization and transcription activity	[103,120–136]
At FKBP53 (similar to Fpr4)	*Arabidopsis*	acidic, basic	H3	histone chaperone activity, rDNA silencing	[220]
Parvulins					
Parvulin	*E. coli*	none	membrane proteins	protein folding / maturation	[27]
Ess1	yeast	WW binds pS/T-P	RNA pol II-CTD, Swi6, Whi5; Rpd3, Spt23	RNA pol II transcription and RNA processing; CTD phosphorylation, nuclear localization of transcription factors; prevents Spt23 ubiquitylation	[29,53,70,94,95,142,153]
Dodo (Ess1 ortholog)	*Drosophila*	WW	CF2, RNA pol II	promotes Ubiq-dependent degradation of CF2; important for pol II function	[88,53]
Pin1 (Ess1 ortholog)	mammals, plants	WW binds pS/T-P	p53, p73, c-Fos, NF-κB, c-Myc, Oct4, FOXO4; RUNX, SLBP, *etc.* RNA pol II-CTD	RNA pol II transcription; signal transduction, transcription factor stability, nuclear import, activation	[19,45,54,55,60–69,76,77,85–88,90,91,112–118,234]
Hs Par14	humans	basic	unknown	pre-ribosomal RNA processing	[20,53]
Orphan					
Rrd1 (Ypa1)	*S. cerevisiae*	hPP2A activator-like	RNA pol II-CTD	binds rapamycin and effects chromatin association of RNA pol II	[154,155]

[1]Most PPIases listed are also conserved in other organisms; [2]Abbreviations are listed in Fig. 2 legend.

its processing, and thus activate the nuclear, transcriptionally active form of Notch [69]. Activation required both the catalytic activity of Pin1 as well as MAPK phosphorylation sites (Ser/Thr-Pro) in the Notch STR domain known to be important for γ-secretase cleavage. The disparity with the prior study [68] has been proposed [69] to be due to increased γ-secretase activity known to occur in p53 knockout mice used in that study. Thus, Pin1 regulates Notch activity through post-translational processing.

In yeast, genetic studies linked the Ess1 isomerase to cell cycle transcription factors Swi6 and Whi5 [70], whose nuclear-cytoplasmic shuttling is regulated by phosphorylation [71–74]. Ess1 is required for nuclear localization of Swi6 and Whi5, and Ess1 binds specifically to phosphorylated peptides corresponding to the NLS of Swi6, and to the NLS and nuclear export sequences (NES) of Whi5 [70], which contain between one and three Ser-Pro binding motifs. As will be described for RNA polymerase II, *cis*/*trans* isomerization of pSer-Pro bonds can stimulate phosphatase activity and it was suggested that Ess1 regulates localization of Swi6 and Whi5 by stimulating their dephosphorylation (a prerequisite for nuclear entry), or by causing conformational changes that affect interactions with nuclear pore complexes (importins and exportins) [70].

Higher plants have expanded repertoires of cyclophilins and FKBPs, many of which are located in the chloroplast, and they contain parvulin-like PPIases [75,76]. *Arabidopsis thaliana* contains at least 29 cyclophilins, 23 FKBPs, and three parvulins [77]. PPIases in plants play a variety of roles in development, flowering, and environmental stress response [78], which as in mammalian cells are typically mediated via effects on signal transduction proteins and transcription factors [79]. Examples include *Arabidopsis* ROF1, an FKBP that activates Hsp90 steroid hormone-receptor complexes as in mammals and may facilitate their nuclear import, [80] (discussed below), and PAS1, which interacts with the transcription repressor FAN in response to auxins and facilitates its nuclear localization [81].

2.2. Stability of transcription factors

One common mechanism of regulation of transcription factors by PPIases is by control of ubiquitin-dependent degradation (reviewed in [51,54,82]). An example in plants is that of cyclophilin Cyp20-2, which appears to promote phosphorylation and degradation of the BZR1 transcription factor, a negative regulator of flowering in *Arabidopsis* [83]. In mammals, Pin1 can stabilize or promote degradation of target proteins. An early example is human Pin1 downregulation of c-Myc. Pin1 binds phosphorylated c-Myc and potentiates its de-phosphorylation by the PP2A phosphatase, thereby promoting ubiquitin-mediated degradation [84]. Similarly, Pin1 binds to Smad2/3 proteins in a phosphorylation-dependent manner and promotes ubiquitin-dependent degradation, thereby attenuating TGF-β signaling [85]. Smad regulation by Pin1 appears to require PPIase activity, as a Pin1 catalytic site mutant (C113A) does not degrade Smad2. Pin1 also promotes the degradation of the normal, but not the disease-associated, epidermal differentiation factor isoform ΔNp63α, a relative of p53 [86], and Pin1 promotes degradation of the mammalian ortholog of the *Drosophila* Runt transcription factor, the RUNX3 tumor suppressor [87].

Perhaps the most physiologically compelling example is in *Drosophila*, where the Ess1/Pin1 ortholog, called Dodo is important for degradation of the CF2 transcription factor during oogenesis [88]. CF2 is a downstream effector in the EGF-R signaling pathway that is initiated by the *gurken* protein, which acts as a region-specific ligand to specify dorsal follicle cell fate [89]. Activation of this pathway results in a single phosphorylation on CF2 at a MAPK site (Thr40-Pro41). When this site was mutated (to Ala40-Pro41), *in vitro* binding by Dodo was reduced, and in S2 embryonic cells, ubiquitylation decreased and CF2 was stabilized. *In vivo*, *dodo* mutants phenocopied both *egf-r* mutants and CF1 overexpression lines, resulting in ventralized embryos, consistent with the idea that Dodo targets CF2 for degradation *in vivo*.

While the above examples confirm a role for Pin1 in promoting transcription factor degradation, there are, in fact, more examples of Pin1 *preventing* degradation by blocking ubiquitylation. Among the mammalian transcription factors reportedly stabilized by Pin1 are NF-κB, p53, c-Jun, c-Fos, Oct4 and Nanog (reviewed by Liou *et al.*, see references therein) [54], and in plants, AGL24, a MADS-domain protein [79]. Pin1 was shown to bind to four phospho-Ser/Thr-Pro sites in p53 and stabilize it following DNA damage [90,91]. This likely requires PPIase activity, as stabilization of p53 *in vitro*, assayed by protease sensitivity, required a catalytically active Pin1. *In vivo*, loss of Pin1 (MEF Pin1 knockout cells) was correlated with increased binding of Mdm2 to p53. Mdm2 is known to promote ubiquitin-dependent degradation of p53 [92]. Thus, Pin1 stabilizes p53 by preventing its interaction with Mdm2 [90]. Curiously, a second PPIase, FKBP25, also stabilizes p53 but does so by promoting Mdm2 ubiquitylation and degradation [93]. Thus both Pin1 and FKBP25 keep Mdm2 from p53, the former via conformational changes in p53, the later via destruction of Mdm2.

In yeast, Ess1 (inappropriately referred to as Pin1 in this study) stabilizes the transcription factor Spt23 by decreasing its levels of polyubiquitylation [94]. In an Ess1 catalytic mutant (H164R) [95] or in "glucose shut-off" strains, reduced Ess1 activity correlated with increased polyubiquitylation and degradation of Spt23. Finally, Ess1 might promote stability of RNA polymerase II itself, as it was shown genetically and by 2-hybrid analysis that Ess1 competes with Rsp5, an E3 ubiquitin ligase, for binding to the large subunit of the polymerase [96]. Similar to the case for nuclear localization, control of transcription factor stability by PPIases is likely to be indirect, via influences on downstream covalent modification (phosphorylation, ubiquitylation). For a more comprehensive discussion control of protein stability by Pin1, see the reviews of Liou et al. and Dilworth et al. [51,54]

2.3. DNA binding and transcriptional activity

While it is often difficult to separate the effects of PPIases on transcription factor stability and translocation from effects on DNA-binding and transcriptional activity, there are examples for each family of PPIase. For the cyclophilins and FKBPs, the most frequently identified regulatory interactions with transcription factors are with nuclear steroid-hormone receptors (discussed in 2.4). However, other DNA-binding proteins are also affected. The earliest example is cyclophilin 40 (Cyp40), which interacts with c-Myb, but not v-Myb, to inhibit its DNA-binding activity in gel-shift assays [97]. Both the TPR motif and the PPIase domain of Cyp40 were required for this inhibition, and cyclosporin A blocked the ability of Cyp40 to inhibit c-Myb DNA-binding. And in an oft ignored control, the authors showed that interaction of Cyp40 with c-Myb is not blocked by the drug interaction. Together, these data indicate the isomerase activity is required. Using a different mechanism of regulation, the single domain PPIase, cyclophilin B (CypB) interacts with the nuclear translocated form of the prolactin receptor to simulate DNA-binding and transcription activation by Stat5. It does so by causing the release the Stat inhibitor PIAS3 [98]. A catalytic mutant of CypB that still interacted with the prolactin receptor was reduced in its activity, suggesting the isomerase activity is important for Stat5 activation, although the exact target of isomerization is not known.

FKBP12 and FKBP25 have been shown to regulate the mammalian YY1 zinc-finger transcription repressor [99,100]. FKBP12 (a single domain PPIase) interacts with YY1 in yeast and mammalian two-hybrid assays, and in transfected HeLa cells, FKBP12 reduced YY1-dependent repression, effectively converting YY1 from a repressor into an activator [99]. The mechanism is not known. FKB25, a multidomain PPIase also interacts with YY1 but does so via its N-terminal domain, not its catalytic domain (Fig. 1). The N-terminus of FKBP25 enhances YY1's DNA-binding activity, and FKBP25 effects on YY1 are not disrupted by FK506 or rapamycin, indicating that isomerization of YY1 is not a likely mechanism [100]. The N-terminal domain, which forms

a 5-helix bundle (BTHB) [101], also confers on FKBP25 the ability to recruit histone deacetylases, HDAC1 and HDAC2, which are important for repression by YY1.

In lymphocytes, FKBP52, a large multidomain PPIase, regulates the interferon regulatory factor IRF-4 [102]. FKBP52 uses its TPR motif to interact with IRF-4 and its prolyl isomerase activity is necessary to inhibit IRF-4 DNA binding and transactivation of target genes. Changes in gel migration and protease sensitivity were detected in IRF-4 *in vitro* upon the addition of FKBP52, consistent with a conformational change, one that did not occur in the presence of PPIase inhibitor ascomycin (FK506 analog), suggesting that isomerization of IRF-4 does occur. Most recently, FKBP51 and FKBP52 have been implicated in regulation of NF-κB at multiple levels – nuclear translocation, DNA binding and transcriptional activity [103]. As in the case of steroid receptors, FKBP51 was inhibitory, while FKBP52 was stimulatory for gene activation by NF-κB. Activities of both FKBPs were TPR-dependent, but did not involve interactions with HSP90. By contrast, PPIase activity was required for stimulation by FKBP52, but not for inhibition by FKBP51, as assayed by FK506 sensitivity. This study reveals that the FKBP51/52 duo exerts concerted control over the biological responses mediated by the NF-κB pathway in a manner analogous to how they control steroid hormone responses (2.4 below).

The greatest number of interactions between PPIases and transcription factors (and signaling proteins) have been reported for the Pin1 isomerase. This is probably because binding sites for Pin1 are easily identified (pSer-Pro or pThr-Pro), and Pin1 shows little preference for flanking residues [23]. In addition, phosphorylation at these sites is carried out by well-studied cyclin-dependent (CDK) and mitogen-activated (MAPK) kinases and is ubiquitous in the proteome. These features prompt many investigators to look *a priori* for interactions with Pin1, so it is perhaps not surprising that many, many targets of Pin1 have been reported [54]. How Pin1 might achieve some degree of specificity (if it does) *in vivo* is still a mystery. Indeed, a recent GST-Pin1 pulldown/mass spectrometry experiment found over 600 Pin1-interacting proteins [104]. For more discussion on the "specificity problem", see articles by Hanes [53] and Lippens [105].

An early and intriguing finding was that Pin1 interacts with the phosphorylated form of human Spt5, which along with hSpt4 constitutes the DSIF (DRB-sensitive inducing factor) complex [106]. DSIF together with NELF (negative elongation factor) are responsible for promoter-proximal pausing of RNA polymerase II in mammalian cells [107,108]. Upon phosphorylation of DSIF and NELF (and the C-terminal domain of Rpb1) by P-TEF-b (Cdk9/CyclinT), NELF is released, and DSIF is "converted" into a positive elongation factor, as in yeast where Spt4/5 facilitates elongation. The nature of this conversion is not known, but could potentially involve conformational changes induced by Pin1-dependent isomerization. In yeast, genetic interactions were detected between genes encoding Ess1 and Spt4/5 and Bur1 (yeast Cdk9), suggesting the regulation of Spt4/5 by Pin1/Ess1 may be conserved [109,110].

Pin1 also appears to regulate of p53 family members by a variety of mechanisms. p53, p63 and p73 and their various isoforms play key roles in development, cell proliferation, metabolic regulation and tumor suppression [111]. Each contains a DNA-binding and transcription activation domain, a proline-rich region and an oligomerization domain. Ser/Thr-Pro sites within the activation domain, DNA binding domain and proline-rich domains (PRD) are targets of Pin1 and mediate different effects [112]. Apart from regulation of protein stability of p53 and p63 described above, Pin1 appears to promote p53 DNA binding in stress-induced cells, as shown by Pin1 RNAi knockdown and p53 chromatin-IP on the p21 promoter [112]. And, as shown for p73, Pin1 promotes the recruitment of the p300 co-activator, which acetylates p53 (and p73) increasing its ability to activate transcription [113]. Finally, Pin1 helps dissociate the apoptosis inhibitory protein iASPP from the PRD domain of p53, thus allowing p53 to regulate pro-apoptotic genes [112]. Thus, Pin1 through a variety of mechanisms, regulates p53 family functions [114].

Similar to its effects on p53 and p73, Pin1 augments DNA binding activity, p300 interactions, and transcription activation by the Stat3 transcription factor following growth factor-stimulated phosphorylation of Ser/Thr-Pro motifs, and in particular the S727-P728 motif in the Stat3 activation domain [115]. Pin1 also binds the transcription activation domain of c-Fos following mitogen stimulation in a phosphorylation-dependent manner and increases c-Fos transactivation by an unknown mechanism [116]. Pin1 may promote DNA binding activity of NF-κB in some tissues. Following liver injury, Pin1 promoted transactivation by NF-κB [117], but did not do so by stimulating nuclear import as reported for other cell types [62]. In fact, nuclear p65 levels increased in Pin1-/- knockout cells. Instead Pin1 stimulated DNA binding activity *in vitro* by NF-κB in Pin1 +/+ hepatocytes [117].

Although Pin1 has previously been shown to promote c-Myc degradation in normal cells [84], recent work shows that Pin1 also promotes c-Myc DNA binding and gene activation, independent of effects on c-Myc levels [118]. This regulation requires Pin1 PPIase activity and phosphorylation of c-Myc on S62-P63, and involves Pin1-dependent recruitment of c-Myc to target gene promoters, along with increased recruitment of co-activators such as p300 and the GCN5 histone acetyltransferase, and the chromatin remodeler SNF5 [118]. Interestingly, Pin1 seemed to stimulate the dynamics of c-Myc's interaction with chromatin (both recruitment and eviction), which may be important for its transactivation function. It was speculated that Pin1-dependent conformational changes in c–Myc's basic helix-loop-helix leucine zipper domain (bHLH-LZ) may regulate its DNA binding activity [118]. This region does have two prolines, but neither is preceded by a Ser or Thr, so any potential Pin-1 induced conformational changes would occur elsewhere in the protein.

2.4. Multiple roles for PPIases in nuclear hormone receptor function

Regulation of nuclear (steroid) hormone receptors by PPIases cannot be neatly broken down into distinct activities. That is because regulation typically involves multiple interdependent effects on steroid binding, nuclear translocation, recruitment of receptors to chromatin, and recruitment of co-activators/co-repressors. And more often than not, while their PPIase catalytic domains are required for function, their isomerase activity *per se* is not. In effect, the large multidomain TPR-containing immunophilins (FKBPs and cyclophilins) function as scaffolds and co-chaperones for the assembly and regulation of Hsp90-receptor complexes. There is an extensive literature on nuclear receptor regulation by FKBP51, FKBP52 and Cyp40 and I refer the reader to the following reviews for more discussion and additional citations [16,52,58,119–122]. Here I will provide a brief overview for how they regulate steroid-hormone receptor activity, highlighting important principles and unknowns.

Earlier models for steroid hormone receptor function suggested that binding of hormone induced a conformational change that released intracellular receptors from Hsp90 (or other heat shock protein chaperones) allowing them to translocate to the nucleus and regulate gene transcription [123]. Currently, it is thought that Hsp90 and immunophilin co-chaperones remain associated with the ligand-bound receptors and function to escort them into the nucleus, whereupon the receptor monomers dimerize, bind DNA, and regulate transcription [124] (Fig. 3). Distinct immunophilin-Hsp90-receptor complexes are formed as a result of competition among different TPR-containing FKBPs and cyclophilins for binding to Hsp90 [26]. Regulation of specific complex formation may underlie developmental and tissue specificity of receptor function, but little is known about how this might occur.

FKBP51 and FKBP52 were the first immunophilins to be identified as members of the steroid hormone receptor complexes [125,126]. These PPIases act, along with Hsp90 and the p23 co-chaperone to control the activity of all the major "type 1" steroid receptors: androgen receptor (AR), estrogen receptor (ER), glucocorticoid receptor (GR), progesterone

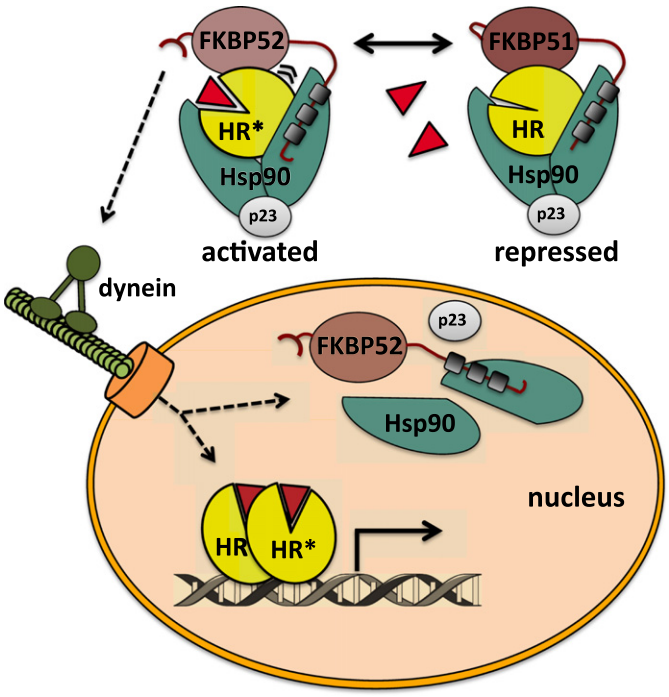

Fig. 3. Schematic of FKBP co-chaperone activity for nuclear hormone receptor complexes. FKPB51 is replaced by FKBP52 in activated receptors (HR*, ligand bound), promoting translocation to the nucleus via interaction with dynein. Once in the nucleus, the complex dissociates, the hormone receptor dimerizes, binds DNA and regulates transcription. HR, hormone receptor; Hsp90, heat shock protein chaperone; p23, small co-chaperone protein. See section 2.4 for details.

receptor (PR), and mineralocorticoid receptor (MR) (reviewed in [120,121]). FKBP51 and FKBP52 use their TPR motifs, and a C-terminal extension to bind directly to Hsp90 within receptor complexes. Regulation also requires an intact PPIase domain, but mutational studies clearly indicate that the isomerase activity is not critical [127]. This serves as a cautionary tale for other studies that the requirement for a PPIase domain does not necessarily mean that prolyl isomerization is important for the observed effects.

In general, FKBP51 is a negative regulator of nuclear hormone receptor activity, while FKBP52 is a positive regulator. An exception is that FKBP51 stimulates AR [121]. Both FKBP51 and FKBP52 contain functional PPIase catalytic domains (FK1) as well as a second domain (FK2) that is non-functional (Fig. 2). And, their TPR motifs are interchangeable [128]. How then does FKBP51 inhibit while FKBP52 activates the same receptors? An early clue came with finding that FKBP51 and FKBP52 exchange during GR receptor activation, with unliganded receptor preferentially associating with FKBP51 [129]. Upon hormone activation, FKBP51 is replaced by FKBP52, which in turn binds the motor protein dynein [130] to help transport the receptor complex into the nucleus. Later work also showed that FKBP52 stimulates hormone binding [131,132], while FKBP51 inhibits hormone binding [129,132,133]. Thus, the current model (Fig. 3) is that the FKBP51-Hsp90 receptor complex is in a repressed state, confined to the cytoplasm. Steroid binding favors the FKBP52-Hsp90 receptor complex which associates with dynein-dynactin and is transported along microtubules to the nuclear pore complex whereupon it is imported into the nucleus. Once in the nucleus, FKBP52 and Hsp90 dissociate from the receptor, which dimerizes, binds DNA and regulates transcription [124,134].

FKBP51 binds poorly with dynein [135], explaining why the repressed complex stays in the cytoplasm. Functional differences between FKBP51 and FKBP52 have been mapped to the C-terminal extension beyond the TPR motifs and a proline-rich loop on the FK1 PPIase domain [127,136], but the exact mechanisms of interaction with dynein and nuclear import are still not clear, nor is the mechanism of "uncloaking" once in the nucleus. Curiously, in cells treated with geldanamycin, which disrupts Hsp90 interactions (and presumably FKBP interactions), receptor dimers still form in the cytoplasm, with or without ligand, but these fail to be imported into the nucleus [124].

Cyp40 is the only cyclophilin that carries TPR motifs (Fig. 2), and perhaps not surprisingly, it binds to Hsp90 and is a member of several nuclear hormone receptor complexes including AR, ER, PR and GR complexes [137,138]. Cyp40 regulates Hsp90 ATPase activity, and like FKBP52, is thought to help increase hormone binding and promote interaction with dynein to promote nuclear localization [139]. Cyp40 targets the receptor complex via TPR-Hsp90 interactions, while a surface loop in the Cyp40 PPIase domain is thought to contact the ligand binding domain of the receptor causing a conformational change that increases its affinity for hormone [140]. Results of experiments in yeast using Cpr7 (a Cyp40 ortholog) and mutants thereof, indicate that the catalytic activity of Cyp40 is not important for receptor activation. In sum, Cyp40 function closely parallels that of the FKBPs in receptor regulation, and is likely to compete with them for incorporation into Hsp90-receptor complexes. Given the widespread role of nuclear hormone receptors in development, physiological responses and disease, understanding exactly how FKBPs and Cyp40 regulate their activity remains an important goal.

3. Control of RNA Polymerase II

The most direct way in which prolyl isomerases affect gene transcription is by targeting the RNA polymerase enzyme itself. In eukaryotes, both cyclophilin and parvulin PPIases bind directly to the carboxy terminal domain (CTD) of Rpb1, the largest subunit of RNA polymerase II (pol II). And, although PPIases do not seem to have a measurable effect on the rate of ribonucleotide addition *in vitro* [44], they are nonetheless important for pol II function *in vivo* [95,141]. Evidence thus far indicates that PPIases act by controlling the ordered assembly of RNA pol II subcomplexes that are necessary for efficient elongation, termination and co-translational RNA processing [95,109,142], (reviewed in [53]).

3.1. PPIases are linked to RNA polymerase II transcription

The first studies linking a PPIase to transcription were those of Hani et al. [18,141]. They found that temperature-sensitive (*ts*) mutations in yeast Ess1 (called *PTF1* in their studies) were defective in 3′-end processing as detected by transcription readthrough of genes with poly(A)-dependent terminators. At the time, this was a curious result because many studies were pushing the idea that Pin1, the human Ess1 ortholog was a "mitotic regulator" whose main targets were cell cycle proteins [19,143]. [*n.b.* This view continues today, with most studies ignoring potential transcriptional consequences of alterations in Pin1]. One study, however, found that Pin1 associated with phosphorylated pol II in HeLa cell extracts and suggested Pin1 might have a regulatory role [144]. Most convincingly, in an unbiased genetic screen, the author's laboratory discovered that a number of transcription-related genes, when present at high-copy, rescued the growth defects of *ts*-mutations in Ess1 [95]. These included *RPB7*, and *FCP1*, which encodes a CTD phosphatase. Moreover, it was shown that $ess1^{ts}$ mutants interact genetically with *RPB1* reduced-dosage and CTD-truncation mutants, and with *SRB2* mutants (encodes a mediator component), and that Ess1 binds phosphorylated pol II via its CTD and affected reporter gene expression [95]. Based on these data, we proposed a model in which Ess1-dependent isomerization of the CTD controls the binding and release of proteins required for RNA pol II function [95] (Fig. 4A). Concurrently, a biochemical fractionation/mass spectrometry study found Ess1 to be among a number of proteins that bound to a phospho-CTD column and this was reported in an accelerated publication [145].

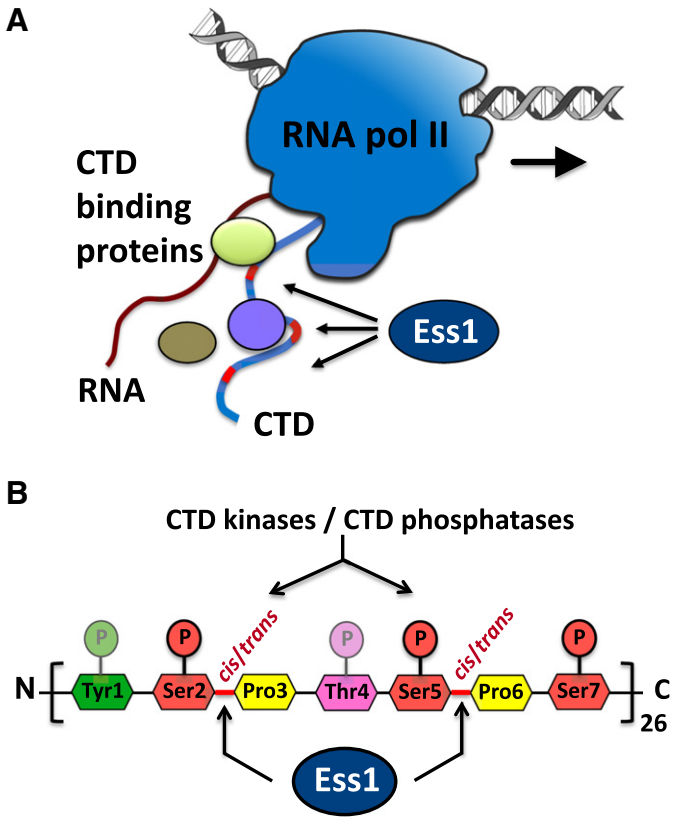

Fig. 4. Role of Ess1 in control of RNA polymerase II and the CTD code. (A) Ess1 binds to the phosphorylated form of the carboxy-terminal domain (CTD) of the Rpb1 subunit. Ess1 induces conformational changes in the CTD that in turn regulate binding of RNA pol II co-factors (CTD-binding proteins) to the transcription complex. CTD-binding proteins (*e.g.* Ssu72, Nrd1, Pcf11, Cgt1) show isomer-specific (*cis vs. trans*) recognition. Ess1 also controls phosphorylation of the CTD (not shown). (B) Potential modifications of the CTD that collectively constitute the CTD code. Phosphorylation occurs at multiple different residues and isomerization occurs at the two proline bonds. The CTD in yeast contains 26 repeats of this sequence. Not shown are potential glycosylation and other modifications that occur on non-consensus sites in mammals. See sections 3.2–3.4 for details.

Cyclophilins also interact with the CTD of Rpb1. Early on, a human multi-domain cyclophilin bearing serine-arginine rich (SR)-repeats, typically found in RNA-splicing proteins, was identified in a two-hybrid screen using a Gal4-CTD bait [146]. The interaction was mediated by the SR-domain but no evidence was provided for a role of the cyclophilin domain. This protein was called SRcyp/CASP10, and localized to the nuclear speckle. It was proposed to target phosphorylated RNA pol II and induce conformational changes in the CTD to regulate RNA splicing [146]. This protein was also identified as human CARS-CYP [147], and as Matrin-Cyp in rat, which was shown to co-localize with splicing factors, have PPIase activity comparable to that of traditional cyclophilins (CypA, CypB), and likely contribute to cyclosporin A sensitivity of nuclear prolyl isomerase activity [148].

In plants, *Arabidopsis* AtCyp59, a multidomain cyclophilin containing an RNA recognition motif (RRM) and an SR-rich region was isolated by two-hybrid interaction with an SR-protein, but it was also shown to interact with the CTD of RNA pol II [149]. Although AtCyp59 is not conserved in *S. cerevisiae*, an ortholog in *Schizosaccharomyces pombe*, Rct1, was shown to be essential for growth and bind to the pol II CTD and downregulate its phosphorylation levels (on Ser2 and Ser5) [150]. This activity is similar to that of Ess1 (discussed below), and suggests that in *S. pombe*, Rct1 may be partially redundant with Ess1 [151]. Rct1 associated with actively transcribed genes (promoter, coding and 3′ regions), and in heterozygous *rct1* mutants nuclear run-on transcription was reduced. This study provides strong evidence that cyclophilins regulate RNA pol II function [150]. How exactly Rct1 recognizes the CTD and whether, like Ess1/Pin1, recognition is phosphorylation-dependent, is not known.

Another plant cyclophilin, CsCyp also interacts with the CTD [152]. CsCyp is a citrus (sweet orange) cyclophilin, related to yeast Cpr1 (CypA), that was shown to mediate the transcriptional response of bacterial pathogens responsible for citrus canker. Citrus CsCyp possesses isomerase activity that is cyclosporin A-sensitive, and when over-expressed, rescues *ess1*^{ts} mutations in yeast (as does yeast Cpr1) [153]. Interestingly, the bacterial pathogenic protein PthA2 binds the plant CsCyp and inhibits its isomerase activity, which probably contributes to the mechanism of transcriptional disregulation and pathogenesis induced by PthA2 (*i.e.* via effects on CsCyp regulation of the CTD) [152].

The yeast protein Rrd1 (*r*apamycin *r*esistant *d*eletion 1), which has similarity to mammalian phosphotyrosyl phosphatase 2A activator protein (hPTPA), has been shown to have prolyl isomerase activity [154]. Rrd1 (also known as Ypa1) interacts with Rpb1 and induces conformational changes to GST-CTD fusion proteins *in vitro* in a rapamycin-dependent manner [155]. ChIP assays indicate that *in vivo*, Rrd1 mediates rapamycin effects on the recruitment of RNA pol II to chromatin, showing differential effects at different loci. In this and a genome-wide study [156], Rrd1 appears to redistribute RNA pol II during conditions of stress, such as rapamycin treatment, and likely does so by changes in phosphorylation of the CTD. In addition, genetic and ChIP data support the idea that Rrd1 functions in pol II elongation, although this remains to be demonstrated [156].

3.2. The CTD as a target for covalent and non-covalent modification: The CTD code

The CTD of RNA pol II is a fascinating domain of the Rpb1 subunit that seems to take on a life of its own. Indeed, it can be transferred onto other subunits (Rpb4 or Rpb6) where it retains its function [157]. A casual inspection of the CTD sequence, (Y-S-P-T-S-P-S)n, immediately reveals why it is of so much interest to prolyl isomerase researchers, particularly those studying Ess1/Pin1 parvulins, whose consensus recognition site is pSer-Pro. In yeast, there are 26 almost identical repeats of this sequence, while in humans there are 52 repeats with additional degeneracy. Prokaryotic and archaeal RNA polymerases do not contain this motif, nor do eukaryotic RNA polymerases I and III. This evolutionary addition to pol II seems to coincide with the increased complexity of transcription regulation and RNA processing of eukaryotic mRNA encoding genes. Perhaps the most interesting structural aspect of the CTD is that, like other low-complexity and intrinsically-disordered domains, it takes on a defined structure only when bound to other proteins. In the case of the CTD, the structures can be very different depending on the binding partner [158–160]. There is strong evidence that the *cis*/*trans* conformations of the resident prolines are a critical feature of the CTD structures as will be discussed below (Section 3.4). A number of outstanding reviews about the evolution, structure and function of the CTD are available [161–173].

The CTD sequence is likely to have been selected for residues with maximum versatility. This short motif can be covalently modified by phosphorylation, primarily at Ser2, Ser5, and Ser7, but also at Tyr1 and Thr4 [174]. In mammals, the Ser and Thr residues can also be glycosylated [175,176], while degenerate Arg7 and Lys7 residues can be methylated [177], or potentially acetylated, methylated, sumoylated or ubiquitylated [166]. Finally, and most importantly for the purposes of this review, the two Ser-Pro bonds can be non-covalently modified by *cis*/*trans* isomerization [32,178]. A summary of these modifications is shown (Fig. 4B).

Collectively, the different patterns of CTD modification are thought to constitute a “CTD code” for recognition and recruitment of proteins to the RNA pol II complex [95,179]. Serine phosphorylation, which is the most easily detected CTD modification due to the availability of phospho-Ser specific monoclonal antibodies [180], shows characteristic

patterns across genes [161,181–184]. Levels of Ser5 and Ser7 phosphorylation are highest at the 5′ ends of genes near the transcription start site, while Ser2 phosphorylation increases over the body of the gene, peaking at the 3′ end near the transcription termination site. Phosphorylation of Ser7 remains high while that of Ser5 diminishes sharply toward the 3′ region. Differential phosphorylation of the CTD is thought to help the ordered recruitment (and eviction) of RNA pol II co-factors necessary during the transcription cycle, *e.g.* for intiation, 5′-capping, elongation, 3′ cleavage of mRNA, and transcription termination [163,164,167]. Little is known about the patterns of other covalent and non-covalent modifications.

3.3. Ess1 and Pin1 are readers and writers of the CTD code

The Ess1 and Pin1 prolyl isomerases function as both readers and writers of the CTD code. They are readers because they recognize and bind only to the phosphorylated forms of the CTD [30–32,178]. They are writers because once bound they are capable of inducing conformational changes in the CTD that mediate downstream effects, such as the binding of CTD-modifying enzymes and co-factors required for RNA pol II function [142,185–187].

As readers, the binding of Ess1 (and Pin1) to the CTD *in vivo* depends on prior action of the CTD kinases [109,187,188] which in yeast include Kin28 (human Cdk7), which phosphorylates Ser5 (and Ser7), and Ctk1 and Bur1 (human Cdk9 and Cdk12) which phosphorylate Ser2 [162,189]. Genetic and biochemical evidence shows that Ess1 binds both pSer5 and pSer2 forms of the CTD, but shows a ~4–5 fold preference for pSer5-CTD [109,178]. Chromatin IP on individual genes [187] shows that Ess1 is bound along the entire length of genes including promoters/5′regions (high pSer5), coding regions (moderate pSer5 and pSer2) and 3′ ends (high pSer2). Genome-wide ChIP studies show that Ess1 localizes most strongly to the central part of genes where both Ser2 and Ser5 are phosphorylated (C. Jeronimo, F. Robert pers. comm.). Pin1 also associates with the phosphorylated form of the RNA polymerase II, and may do so in a cell-cycle regulated manner [186,190]. Thus, Ess1 and Pin1 are likely recruited to actively transcribed genes via phospho-CTD interactions.

As writers of the CTD code, Ess1 and Pin1, once bound, regulate the interaction of the CTD with other proteins including CTD phosphatases and kinases [142,187,190]. They do so by increasing the rate of *cis-trans* isomerization of the CTD, which in the context of intact proteins *in vivo* is probably very low. *In vitro*, Ess1 stimulates the prolyl isomerization of phosphorylated-CTD peptides from a spontaneous rate of less than 1 turnover per minute to >1000/min for a pSer5 peptide and ~200/min for a pSer2 peptide [178]. The key here is that PPIases, which catalyze the reaction in both directions (*cis*>*trans* and *trans*>*cis*) and therefore cannot change the overall equilibrium, still have profound kinetic consequences on coupled reactions that involve enzymes or CTD-binding proteins that are specific for *cis* or *trans* substrates.

The most concrete example of how an isomerase can affect the direction of a physiological reaction is that of Werner-Allen et al. [191], who showed that Ess1 stimulated the CTD phosphatase activity of Ssu72, which prefers a *cis*-substrate [191,192]. In solution the low percentage of *cis*-isomer available (~12% of total) was rate-limiting for Ssu72 phosphatase activity. Addition of Ess1 provided a pronounced kinetic advantage, stimulating the phosphatase activity of Ssu72 on both a small CTD peptide and on a "full-length" GST-CTD fusion protein (26 repeats). This stimulation was saturable as expected and required isomerization, since catalytic mutants (C120S, S122P, H164R) [178] did not have any effect [191]. While it is not known what percentage of CTD proline bonds are in the *cis* or *trans* configuration *in vivo*, these studies illustrate how *cis-trans* interconversion by Ess1 or other PPIases can provide a kinetic advantage to an isomer-specific enzyme. Indeed prior genetic and molecular studies showed that Ess1 controls the phosphorylation status of the CTD; Ess1 reduces pSer5 levels by assisting Ssu72, which is a Ser5-specific phosphatase [142,184]. Pin1 also regulates the phosphorylation status of the CTD and appears to affect the function of another CTD-phosphatase, Fcp1, as well as the function of Cdk2 kinase [190].

3.4. Cis/trans specificity of CTD-binding proteins provides a mechanism for prolyl isomerase regulation of RNA polymerase II activity

The structures of almost a dozen CTD-binding proteins have been determined (reviewed in [158–160]). Of these, most, including Cgt1 (capping enzyme) and Pcf11 (3′ processing factor) bind phosphorylated CTD-peptides in which the prolines are found in the *trans* configuration [193–195]. In contrast, *Drosophila* and human Ssu72 bind phospho-CTD peptides in the *cis* configuration, as does the termination factor Nrd1 [191,192,196]. The high selectivity of these proteins for *cis vs*. *trans* isomers supports the idea that the CTD isomerases, including the parvulins Ess1 and Pin1, the cyclophilins SR-cyp, AtCyp59/Rct1, and CsCyp, and the orphan isomerase, hPTPA/Rrd1/Ypa1 play important roles in RNA pol II regulation. In support of this idea, a number of transcriptional defects are observed in *ess1* mutants and Pin1 knockdown or knockout cells (reviewed in [53,57]).

The best examples of the effects of PPIases on transcription are found in yeast. In *ess1*^{ts} mutant cells, even at permissive temperature, widespread transcription readthrough occurs [95,110,142,185,187]. Specifically, readthrough is detected on all independently-transcribed (by pol II) small nucleolar RNA (snoRNA) genes [142]. Importantly, snoRNA termination is guided by Ssu72, Nrd1 and Pcf11, three of the RNA pol II co-factors mentioned above, whose CTD-binding structures show *cis* or *trans* specificity (as well as phospho-Ser preferences). Ssu72 binds and dephosphorylates the *cis*-form of pSer5-CTD. Nrd1 binds to the *cis* form of phosphorylated Ser5-CTD, while Pcf11 binds the *trans* form of Ser2-CTD. Based on genetic, molecular and ChIP data, Ess1 was found to augment Ssu72 function *in vivo*, and to be required for Nrd1-dependent termination. Ess1 functions by coordinating the competition for CTD-binding by Nrd1 and Pcf11 at the 3′ ends of snoRNA genes [142]. These results provide strongest support to date for a role of isomerases in implementing (writing) the CTD code to direct pol II transcription.

It is estimated that as many as half of all mRNA genes require Ess1 for efficient poly(A) dependent 3′ processing and/or termination [187]. The detection of aberrant readthrough and fusion transcripts is facilitated by the use of RNA decay-deficient backgrounds such as *upf1* [187]. The mechanism of Ess1 in poly(A)-dependent 3′ end formation is unknown but likely involves Ess1 coordinating the recruitment of 3′-processing and/or termination factors. Determining their *cis*/*trans* specificity is of obvious interest. Ess1 is also required to suppress genome-wide expression of cryptic unstable transcripts (CUTs) [197,198] and stable unannotated transcripts (SUTS) [199] in intergenic regions and within coding regions in both sense and antisense orientations [142]. In human cells, Pin1 overexpression *in vitro* and *in vivo* was reported to inhibit transcription and mRNA splicing [186]. The effects on transcription are not well studied for the other classes of PPIases although there are clearly transcription-related defects in mutants of Rct1 (cyclophilin) and Rrd1 (orphan PPIase) [150,156].

3.5. The Traffic Cop model for Ess1 control of RNA polymerase II

Studies on Ess1 and Pin1 lead to a general "Traffic Cop" model of how isomerases control transcription and co-transcriptional recruitment of pol II co-factors during the transcription cycle [53]. Ess1 (and other PPIases that target the CTD) change the conformation of the RNA pol II CTD by isomerizing prolyl bonds at Ser2-Pro3 and Ser5-Pro6. *The conformational changes induced by Ess1 coordinate the flow of traffic of CTD binding proteins*. There is probably not an absolute requirement for Ess1 at any given step, as proteins would eventually bind and release from the CTD, perhaps in response to spontaneous *cis*/*trans* interconversion. However, Ess1 would help make each step more efficient, which

would explain why higher levels of Ess1 are needed under conditions of stress [178]. For example, termination of snoRNAs and mRNAs still occurs correctly ~80% or more of the time, and transcription *in vitro* occurs without Ess1/Pin1 [44,110,142,187]. However, without Ess1, the inefficiencies in the transcription cycle, the aberrant RNAs produced, and cryptic transcription that occurs, probably combine to lead to cell death, at least in yeast. In organisms where Ess1/Pin1 is not essential, the defects may be better tolerated or there may exist compensatory mechanisms such as higher expression of other CTD-binding PPIases [151,152] However, transcriptional inefficiencies could also contribute to the developmental and disease phenotypes observed in animals that over- or under- express Pin1 [82,200–202].

4. Control of chromatin structure and histone modification

Prolyl isomerases play a number of distinct roles in gene regulation through alterations in chromatin structure and function [51,203]. These can be categorized as follows (1) controlling the recruitment or activation of histone modifying enzymes and chromatin remodelers (2) functioning as histone chaperones, (3) directly isomerizing histones, in particular the N-terminal tail of histone H3, and least well characterized, (4) a direct or indirect role in higher-order stucture, chromatin compaction and silencing. Teasing out which nuclear PPIase has which activity will be a challenge. In addition, some PPIases carry out multiple distinct functions, such as yeast Fpr4, an ortholog of mammalian FKB25, which functions as a histone chaperone, a scaffold for assembly of chromatin modifying complexes, and a direct histone H3 isomerase, and Cyp33, which binds RNA, targets a histone modifying enzyme (MLL1) and isomerizes histone H3 [51]. In this regard, the isolation of allele-specific mutations should help in the study of individual functions of a given PPIase [41].

4.1. PPIases and histone modifying enzymes

PPIases can affect transcription factor activity by regulating interactions with co-activators and co-repressors, such as histone acetyltransferases (HATs), histone deacetylases (HDACs), and histone methyltransferases (HMTs). Examples mentioned above (Section 2.3) included FKBP25 and its role in recruiting HDACs to the YY1 transcription factor, and Pin1, which recruits the p300 (HAT) co-activator.

PPIases can also influence the assembly and activity of histone modifying complexes. Perhaps the best understood example is that of Cyp33 in regulation of the MLL1 (mixed lineage leukemia protein 1) histone H3K4 methyltransferase [204]. Cyp33 limits the activity of MLL1 complex and effectively converts it from playing an activating role into a playing repressing role. Cyp33 is a multidomain PPIase containing an N-terminal RRM (RNA binding domain) and a C-terminal isomerase domain. In *Drosophila*, Cyp33 interacts with Trithorax, the MLL1/Set1 ortholog, which trimethylates histone H3 (H3K4me3) and downregulates the homeotic gene *AbdB* [205]. Likewise, in mammalian cells Cyp33 binds MLL1 and downregulates transcription of *HOXC8* (ortholog of *Drosophila AbdA*) and *HOXC9* (ortholog of *AbdB*) [206]. Curiously, Cyp33 upregulates *HOXC6* (ortholog of *Antp*, a more anterior gene). Cyp33 uses its RRM domain to interact with the third PHD finger of MLL1 [206] and potentiates the recruitment of HDAC1 to the adjacent repression domain in MLL1 [207]. The effects of Cyp33 on *HOX* gene expression require both the RRM and the isomerase domains, and are sensitive to cyclosporin A, suggesting the catalytic activity is required.

Two outstanding structure-function studies revealed the detailed mechanisms of action of Cyp33 on MLL1 [208,209] (summarized in Fig. 5). The structures of both the RRM domain of Cyp33 and the PHD3 of MLL1 were determined, and the surfaces and residues responsible for their interaction were mapped by NMR and mutagenesis. PHD domains are known to bind H3K4me3 residues [210]. Both studies found that H3K4me3 peptides and the RRM of Cyp33 bind to different surfaces on the PHD3 domain of MLL1. Competition for binding between H3K4me3 peptides and the RRM reduced affinity of the other by only 2-5 fold. Here the studies differed in interpretation; Park et al. [208] concluded that binding was mutually exclusive (they observed 5-fold inhibition), while Wang et al. [209] concluded that simultaneous binding of both the H3 peptide and the RRM of Cyp33 to the MLL PHD domain was permissible (observed 2-fold inhibition).

A key difference in these studies was that Wang et al. [209] used a larger fragment of MLL1, which included the PHD3 domain and its adjacent Bromo domain (which binds acetylated histones). This proved to be incisive, because at the junction between the domains resides a conspicuous proline (Pro1629) that is found in the rare *cis* conformation. They discovered that in the *cis* configuration, the Bromo domain blocks the surface bound by the RRM domain, but does not block the surface binding the H3K4me3 peptide (Fig. 5). Addition of intact Cyp33 (catalytically active) allowed RRM binding, but addition of the RRM alone or a catalytically-deficient Cyp33 did not. Remarkably, a P1629A mutation, which should generate a *trans* conformation, allowed binding of the isolated RRM domain, consistent with the model that Cyp33 isomerization of the His1628-Pro1629 bond changes the conformation of the MLL1 PHD3 domain to allow simultaneous binding to methylated histone H3 and the RRM of Cyp33 to form a stable ternary complex. Wang et al. [209] further postulate that this complex prevents further "spreading" of the H3K4me3 mark by blocking MLL1 Set-domain dependent methylation, and helps to recruit HDAC1, together contributing to transcription repression. Indeed, both groups showed that Cyp33-dependent repression was accompanied by reductions in H3K4me3 and H3 acetylation. Park et al. [208] also proposed that Cyp33 directly isomerizes histone H3. How exactly Cyp33 interaction with MLL1 at target genes is regulated remains unknown, but it may involve RNA binding via the RRM domain.

Additional examples of PPIases affecting the activity of histone-modifying complexes include yeast Ess1 and Cpr1, which interact genetically and physically with members of the Sin3-Rpd3 HDAC complex [153]. Ess1 appears to inhibit Rpd3 function and increases silencing at the rDNA locus, while Cpr1 regulates Rpd3 complex assembly, and decreases silencing at the rDNA locus [153]. Ess1's role in chromatin modification is under study, and initial studies indicate that Ess1 it is also important for H3K4 trimethylation, as double mutants with Set1 (H3K4 methyltransferase) are synthetic lethal, and H3K4me3 levels are diminished in $ess1^{ts}$ mutants [187]. For Cpr1, which is best known as the cytoplasmic target of the cyclosporins, its nuclear function is intriguing. Cpr1 localizes to the nucleus and is required for induction of meiosis-specific genes, *IME1* and *IME2*. Sporulation can be inhibited by cyclosporin A or by mutations that abolish Cpr1's catalytic activity, indicating the PPIase activity is necessary for its nuclear function. Cpr1 is also found in complex with the Set3C HDAC complex [211,212]. A proposed model is that Cpr1 induces conformational changes in the Sin3-Rpd3 complex to relieve repression of *IME2* by Ume6 (converting it from a repressor into an activator), while inhibiting Set3C-dependent repression of *IME1*, *IME2* and other meiosis-specific genes during sporulation [211]. Thus, Cpr1, a single domain PPIase has both nuclear and cytoplasmic functions and is critical for developmental gene regulation.

In mammalian cells, Pin1 is reported to promote dephosphorylation of the linker histone, H1, which would in turn regulate the higher order structure of chromatin [213]. A number of *in vitro* and *in vivo* experiments are suggestive of a model in which Pin1 is recruited to chromatin independent of its interactions with the RNA pol II CTD, and by promoting dephosphorylation of pSer-Pro sites in H1, Pin1 and stabilizes H1's association with chromatin to promote condensation.

In plants, the *Arabidopsis* multidomain cyclophilin AtCyp71, which bears four N-terminal WD40 repeats localizes to and represses homeotic genes including *KNAT* and *STM*. AtCyp71 binds histone H3 and is required for placing H3K27me3 repressive marks on target genes [214]. AtCyp71 likely works by a mechanism that involves recruitment of LHP1, a HP1-like chromodomain protein that is a subunit of a

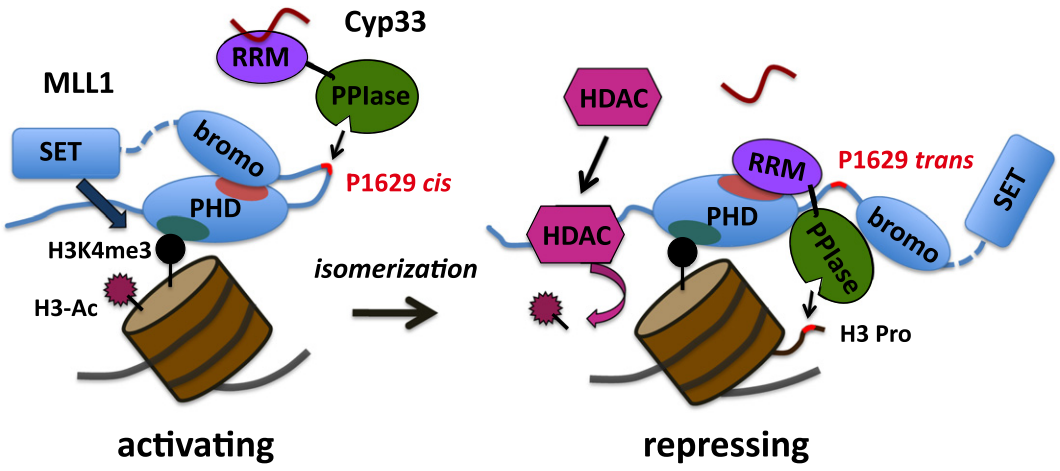

Fig. 5. Cyp33 regulation of the mammalian MLL1 histone methyltransferase protein. The MLL1 complex (along with WRAD proteins [234], not shown) trimethylates histone H3 lysine 4 (H3K4). Cyp33 isomerizes Pro1629 in MLL1 causing MLL1 to rearrange to allow binding of the Cyp33 RRM domain and preventing further methylation via the SET domain. This rearrangement may also assist in the recruitment of histone deacetylases (HDAC). Stable binding of Cyp33 via the RRM may permit further isomerization activity of the PPIase domain, perhaps on histone H3. Together, these changes convert the MLL1 complex from an activating mode to a repressing mode. See section 4.1 for details.

Polycomb-like repressor (PRC-1) complex, which binds to H3K27 methylation marks [215]. How exactly AtCyp71 promotes the deposition of these marks (placed by a PRC2-like complex) is not yet clear. Interestingly, AtCyp71 also interacts with FAS1 protein, a member of a histone chaperone complex, suggesting perhaps that AtCyp71 might help place pre-methylated histones into chromatin [215].

4.2. Histone chaperone activity

A number of nuclear-localized PPIases bind directly with histones and have histone chaperone activity. Histone chaperones facilitate the exchange of canonical histones (H2A, H2B, H3 and H4), and histone variants (*e.g.* H2A.Z) during the assembly and disassembly of nucleosomes, for instance during DNA replication or transcription by RNA polymerases [216]. Two yeast PPIases, *S. pombe* FKBP39 and its *S. cerevisiae* ortholog, Fpr4 were the first demonstrated to have histone chaperone activity *in vitro* [217]. Both proteins localize to the nucleolus and Fpr4 was shown to bind to the rDNA locus and be important for rDNA silencing [217]. Fpr4 histone chaperone activity *in vitro* activity is similar to that of acidic nucleosome assembly factor (NAP1), binding to both H2A-H2B and H3-H4 complexes, with a preference for the latter [218]. The chaperone activity maps to the N-terminal highly-charged nucleolin-like domains of FKBP39 and Fpr4 and does not require the C-terminal isomerase domain [217,218]. In fact, the isomerase domain inhibits chaperone activity, but not by a mechanism that requires catalytic activity [218]. The isomerase domain is important, however, for rDNA silencing *in vivo* [217]. It is likely that Fpr4 contributes to rDNA silencing by the creation of a repressive nucleosome structure via its histone chaperone activity, and potentially by direct isomerization of nucleosomal histones (See section 4.3).

The plant AtFKBP53 is also a histone chaperone and shows structural and functional similarities with Fpr4. AtFKBP53 facilitates nucleosome assembly *in vitro* via an N-terminal acidic domain, interacts preferentially with H3, and localizes to rDNA and promotes silencing [219]. The mammalian FKBP25 protein, lacks the N-terminal acidic domain, but interacts with nucleolin, which shows similarity to the N-termini of Fpr4 and AtFKBP53, and may play an analogous role [203]. The exact mechanism(s) by which PPIases facilitate nucleosome assembly is not known, and is confounded by the fact that, in general, the isomerase activity, which would provide a logical mechanism for influencing histone dimerization or other higher-order assembly, is not required.

4.3. Targeted isomerization of the H3 histone tail

Perhaps in a fitting tribute to the age-old rivalry between Cambridge and Oxford Universities, groups at these institutions discovered that prolyl isomerization regulates H3 lysine modification [220], and conversely that H3 lysine modification regulates prolyl isomerization [221] (summarized in Fig. 6A, and B, respectively). These interesting studies demonstrate crosstalk between covalent and non-covalent modifications of the histone H3 tail in the regulation of gene expression. In the Cambridge study, one of the earliest and most influential papers in the PPIase-chromatin literature, Nelson et al. [220] showed that yeast Fpr4 binds directly to histones H3 and H4 and isomerizes prolines in their amino terminal tails. Isomerization of H3 P38 in turn regulates lysine methylation at nearby H3K36.

Fpr4 was shown to bind H3 and H4 via its N-terminal nucleolin-like domain. Fpr4 recognizes a short 8 residue sequence upstream of the 3 prolines in the H3 tail (P16, P30, P38) and one proline in the H4 tail (P32). Thus, unlike Ess1 and Pin1, recognition is not mediated by the substrate Pro sites themselves but instead by a nearby sequence. Using the standard protease-coupled assay [1], Fpr4 was shown to isomerize the peptide bonds at P30 and P38, but not P16 in H3 and only marginally at P32 in H4. Later, it was shown that the substrate design used for the assay, which requires a C-terminal chymotrypsin site and chromophore (XPF-pNA) [6], influences the result. Using peptides containing only natural H3 sequences (and an NMR-based assay), they showed that Fpr4 catalyzes the isomerization of the P16 and P30 bonds at least three-fold better than the P38 bond [222]. Importantly, Nelson et al. [220] showed that addition of Fpr4 to histone H3 peptides or to intact nucleosomes slowed the kinetics of H3K36 methylation by Set2, but did not affect methylation at other sites (*e.g.* H3K79). Inhibition of Set2 methylation of H3K36 was dependent upon the catalytic activity of Fpr4 and the Pro residue at position 38, suggesting that conformational changes in the H3 tail could regulate this covalent modification.

In vivo, Fpr3 and Fpr4 (which contain nucleolin-like domains) localized to chromatin, but Fpr1 (single domain PPIase) did not. H3K36 trimethylation is typically elevated in the coding region of expressed genes [223]. Deletion and catalytic-deficient *fpr4* mutants did not show increased levels of H3K36me3 on active genes, but did so on uninduced genes. The authors found that Fpr4 activity was important for rapid induction of gene transcription and hypothesized that Fpr4 keeps H3K36 methylation levels low until induction. Isomerization at H3 P38 could potentially affect recognition and/or catalytic activity of

the Set2 methylase and the corresponding Jhd2 demethylase on the nearby K36 residue. Analysis of co-crystals of the human JMJD2A demethylase with a histone H3 peptide reveal the Lys37-Pro38 bond to be in the *trans* configuration, and a P38A mutation does not affect in JMJD2A's H3K36me3 demethylation activity [224]. Therefore, potential *cis*/*trans* specificity would likely reside in Set2 binding and/or methylation activity.

The Oxford group also found isomerization at H3 P16 is important, but that it is regulated by nearby K14 acetylation [221]. They conducted a series of clever experiments making use of both point mutations in histone H3 in yeast, and antisera they developed against *cis*- and *trans*-locked H3 peptides (containing *cis*- or *trans*-hydroxylated prolines). With these in hand, they carried out Western, ChIP, and genome-wide ChIP-sequencing to examine the detailed relationships between acetylation at H3K14, isomerization at Ala15-Pro16, and trimethylation of H3K4. They found that among a set of metabolically and stress induced genes, acetylation of H3K14 promotes a *trans* conformation at Ala15-Pro16 that in turn *decreases* trimethylation at H3K4. This regulatory crosstalk did not apply to other classes of genes, for example ribosomal protein genes, or genes highly expressed during the oxidative phase of the yeast metabolic cycle [225]. They further show that the decrease in H3K4me3 results from a reduced recruitment to chromatin of Spp1, a component of the Set1 (H3K4 methylase), to the 5′ end of genes, and to an increase in activity of the Jhd2 demethylase [or potentially its binding; recall that the human JMJD2A structure [224] mentioned above contains a H3 peptide with a Pro in the *trans* conformation, consistent with these findings].

Taking advantage of the *trans*-specificity of chymotrypsin cleavage in a chromogenic assay, they found that acetylation of K14 or mutation to K14Q (Ac-mimic), but not to K14R (de-Ac mimic) increased the initial rate of cleavage of a H3 peptide substrate, consistent with these modifications promoting a shift toward the *trans* conformation. K14 modifications that alter the *cis or trans* isomerization states at Ala15-Pro16 also regulate effector protein binding to a downstream acetylated lysine motif (K18Ac). Specifically, the Bromo domain of Spt7 binds K18Ac only when the Ala15-Pro16 is in *trans* (*i.e.* when K14 is acetylated, or if Pro16 is replaced by Val, which favors the *trans* conformation). Thus, prolyl isomerization in the H3 tail is regulated in response to covalent modification at K14, which in turn has gene-specific consequences for additional histone modification (H3K4me3) and effector recruitment (to K18Ac). It will be interesting to determine whether the prolyl-isomerase activity of Fpr4 directed at Ala15-Pro16 reciprocally affects K14 acetylation, or possibly increases the sensitivity of the K14Ac - H3K4me3 switch by accelerating the interconversion at A15-P16.

5. Other gene regulatory functions

PPIases have also been implicated in processes that are linked directly or indirectly to transcription, such as RNA splicing [226], mRNA decay [227], DNA repair [104], rDNA silencing and ribosome assembly [228]. Moreover, a number of PPIases contain RNA-binding domains such as the RRM, that may help localize PPIases or regulate their activity. A

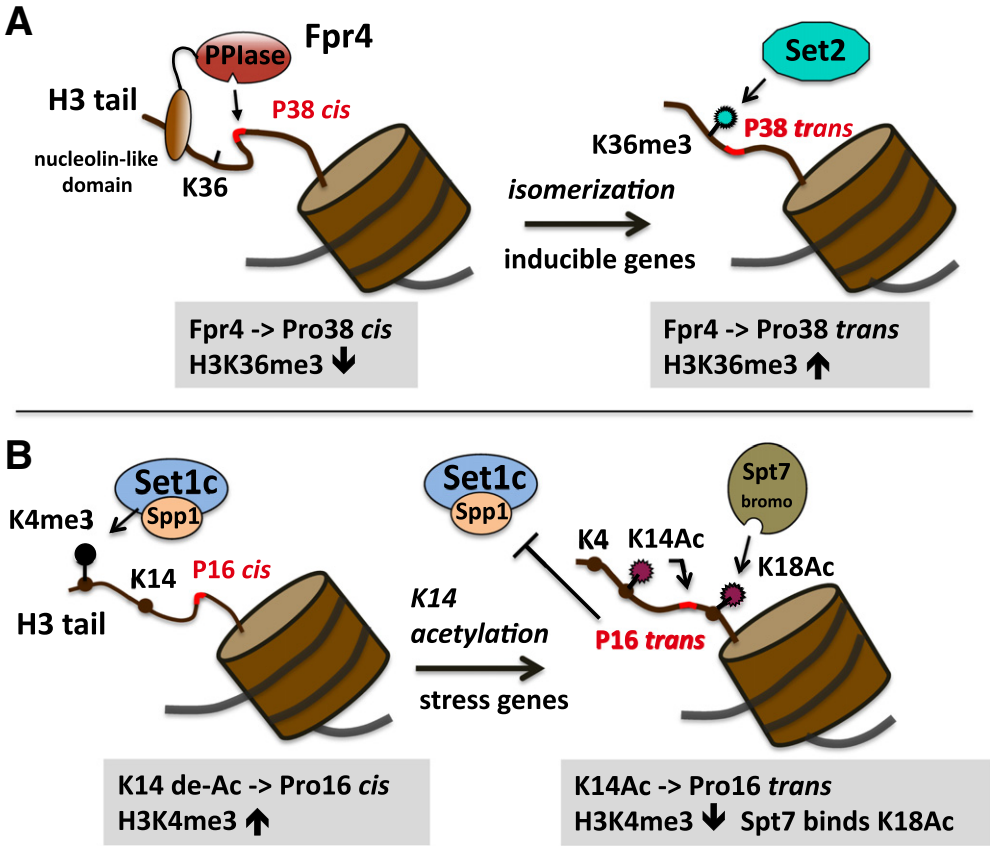

Fig. 6. Prolyl isomerization regulates histone H3 tail modification and *vice-versa*. (A) Fpr4 directly targets prolines in the histone H3 tail. Fpr4 binds via its nucleolin-like domain to the H3 (and H4, not shown) N-terminal tail and isomerizes P16 and P30 (not shown) and P38. Isomerization of P38 is necessary for Set2-dependent methylation at H3K36, at least on highly-regulated genes. Thus, PPIase-dependent isomerization, a non-covalent modification, regulates methylation, a covalent modification. (B) Acetylation controls prolyl isomerization. Acetylation of histone H3 at K14 causes the bond at Ala15-Pro16 to favor the *trans* conformation. This seems to occur without the need for an isomerase enzyme, at least *in vitro* (not shown). The *trans* form of P16 in turn prevents methylation at H3K4, at least in part by preventing recruitment of the Set1 complex (via its Spp1 subunit), and possibly by favoring recruitment or activity of the Jhd2 de-methylase (not shown). The *cis* state of P16 is refractory to binding of Spt7 (a SAGA histone acetyltransferase component) to nearby K18Ac, whereas the *trans* P16 allows binding. See section 4.3 for details.

few examples are given below. Further information and references can be found in other reviews [226,229–231]

5.1. PPIases are regulated by RNA

The *Arabidopsis* AtCyp59 protein discussed in Section 3.1, which is highly conserved from *S. pombe* (Rct1) to humans, contains an RRM that selectively binds to a seven nucleotide motif in RNA (G [U/C]N[G/A]CC [A/G] that is found predominantly in exonic sequences of protein-coding genes [232] and is conserved in *S. pombe*. In plant cell cultures, RNAs containing this motif can be co-immunoprecipitated with AtCyp59, but control RNAs in which this site was mutated cannot. Most interesting was that the isomerase activity of AtCyp59 is inhibited by the addition of motif-containing RNA oligonucleotides (or poly(A) + RNA), but not by control RNA oligonucleotides [232]. Thus, AtCyp59 and its orthologs are RNA-regulated PPIases. Given that *S. pombe* Rct1 ortholog targets the RNA pol II CTD, it is possible that AtCyp59/Rct1 controls the activity of elongating polymerases via its CTD-interaction, and that binding to the nascent RNA transcript would reduce its PPIase activity, thus attenuating its effect(s). Regulation by RNA binding is also likely for Cyp33 in its control of MLL1 [204,205], as discussed (Section 4.1). How RRM-RNA interactions control the activity of PPIase domains remains a mystery.

5.2. PPIases are linked to ribosome maturation and rDNA silencing

In human cells, FKBP25, which regulates YY1 transcriptional activity, was also found in the nucleolus and mass spectrometry analysis identified nucleolin, an RRM-containing protein with a role in ribosome maturation, as a major interacting protein [228]. FKBP25 interactions with nucleolin are strengthened by rRNA and FKBP25 associates with pre-60S ribosomal subunits, but not mature ribosomes, suggesting a role in ribosome biogenesis, perhaps by regulating ribosomal protein folding [228]. In yeast, Fpr3 and Fpr4 play a role in suppressing RNA polymerase II activity in the non-transcribed spacer region (NTS) within the rDNA locus. Silencing by Fpr3 and Fpr4 seems to occur by a mechanism that involves compaction of chromatin, and requires PPIase activity and potentially the isomerization of histone H3 prolines (C. J. Nelson, pers. comm.).

5.3. PPIases control mRNA turnover

In addition to acting on the RNA pol II CTD to influence mRNA production, human Pin1 may act later to regulate turnover of specific mRNAs. Pin1 was shown to affect the stability of histone mRNAs, which have short half-lives and are transcribed by RNA pol II but not polyadenylated [233]. Pin1 targets stem-loop binding protein, SLBP, which binds a 3′UTR structure in histone mRNAs and is important for their maturation and function. Pin1 binds the pThr171-Pro172 site within the RNA-binding domain of SLBP, promoting its dephosphorylation by PP2A and causing the release of histone mRNAs, which are then rapidly degraded. In addition, Pin1 targets other sites in SLBP to promote ubiquitin-mediated degradation. Thus, Pin1 causes decay of histone mRNAs. Pin1 may also control the stability of poly(A)-containing mRNAs. A microarray analysis after Pin1 knockdown identified sets of genes that seem to be co-regulated and that carry common 3′ AU-rich regulatory elements [227]. In addition, Pin1 coimmunoprecipitated with two RNA-binding proteins, AU-rich binding factor 1 (AUF1) and HuR, known to control mRNA turnover [227]. Although additional mechanistic studies are needed, particularly to rule out effects of Pin1 knockdown on transcription rates of the genes identified, it likely that Pin1 controls the stability and RNA-binding avidity of proteins involved in RNA turnover.

6. Summary and future challenges

PPIases control gene transcription in eukaryotes at multiple levels, as summarized in Fig. 7. Regulation can begin at the cell periphery with cleavage of membrane-tethered transcription factors like Notch. In the cytoplasm, PPIases target a host of transcription regulators, from Swi6 in yeast to NF-κB in humans, to regulate their import into the nucleus. PPIases control transcription factor fate by stabilization (p53) or degradation (c-Myc), typically via control of ubiquitin-mediated proteolysis. PPIases work as co-chaperones for steroid hormone receptors to regulate their ligand-binding activity and nuclear import, and once in the nucleus, PPIases control their DNA-binding and transcriptional activity. PPIases also target RNA polymerase II via interaction with the Rpb1-CTD. Isomerization of prolines is part of a CTD code for regulating the activity of RNA pol II via the recruitment of co-factors required for co-transcriptional RNA processing. PPIases also control mRNA processing (*e.g.* 3′-end formation, splicing) and turnover. Finally, PPIases influence transcription by altering chromatin structure and function via a variety of mechanisms that include the recruiting of chromatin and histone modifying enzymes, acting as histone chaperones, and directly isomerizing prolines in the histone H3 tail, which can influence covalent modification of nearby residues, and vice-versa. Finally, PPIases may influence higher-order chromatin structure with effects on gene silencing.

While it has become clear that prolyl isomerases are involved in nearly all aspects of gene transcription (and cell metabolism in general), in most cases, detailed mechanistic information is lacking. This stems from a number of current limitations. First, the fact that PPIases carry out a non-covalent modification (in both directions) makes it difficult if not impossible to track their actions *in vivo*. Thus, functional studies are especially challenging. Indirect readouts are useful, such as monitoring the effects of isomerization on downstream events, such as covalent modification (easier to measure) by enzymes that possess intrinsic isomer preferences (*e.g.* Ssu72 CTD phosphatase). Structural and biochemical studies of additional enzymes – CTD kinases, phosphatases, histone methylases, demethylases, acetylases, deacetylases *etc*., to determine if they have isomer-specific requirements, will be extremely useful for understanding coupled reactions and the importance of PPIase activity in a particular pathway. The use of *cis* or *trans*-specific antibodies is promising, but this approach needs further development, such as the generation of monoclonal antibodies, and accurate detection of *cis* and *trans* forms *in situ* as well as in biochemical assays.

A second limitation in determining detailed mechanisms of action is that the specificity of PPIases remains poorly understood. For example, do the interactions detected by two-hybrid, GST-pull-downs and immunoprecipitations, the most popular approaches, actually reflect the *in vivo* situation? If they do, then PPIases would function rather non-specifically. If not, how are interactions *in vivo* restricted? Here, genetics will help, especially the use of allele-specific and compensatory mutations. In addition, parallel studies in different organisms will help identify conserved interactions that are more likely to be important. For FKPBs and cyclophilins, particularly those that use TPR or other motifs outside the PPIase domain to bind target proteins, defining their actual Pro-containing substrate sites remains an important goal.

A third limitation is that PPIases can have multiple functions. Thus, it is crucial that the nature of the mutant alleles and potential off-target and pleiotropic effects are considered. For example, interpreting the effects of knockout alleles for example in MEF cells (-/-), or siRNA knockdown for a given PPIase (say Pin1) can be tricky due to pleiotropic effects on gene expression, including but not limited to defects in transcription factor stability and localization, RNA pol II CTD regulation, mRNA processing and turnover, and cell cycle effects to name a few.

Finally, and curiously, the actions of PPIases do not always require their enzymatic activity. In many cases, PPIases act simply as proline-

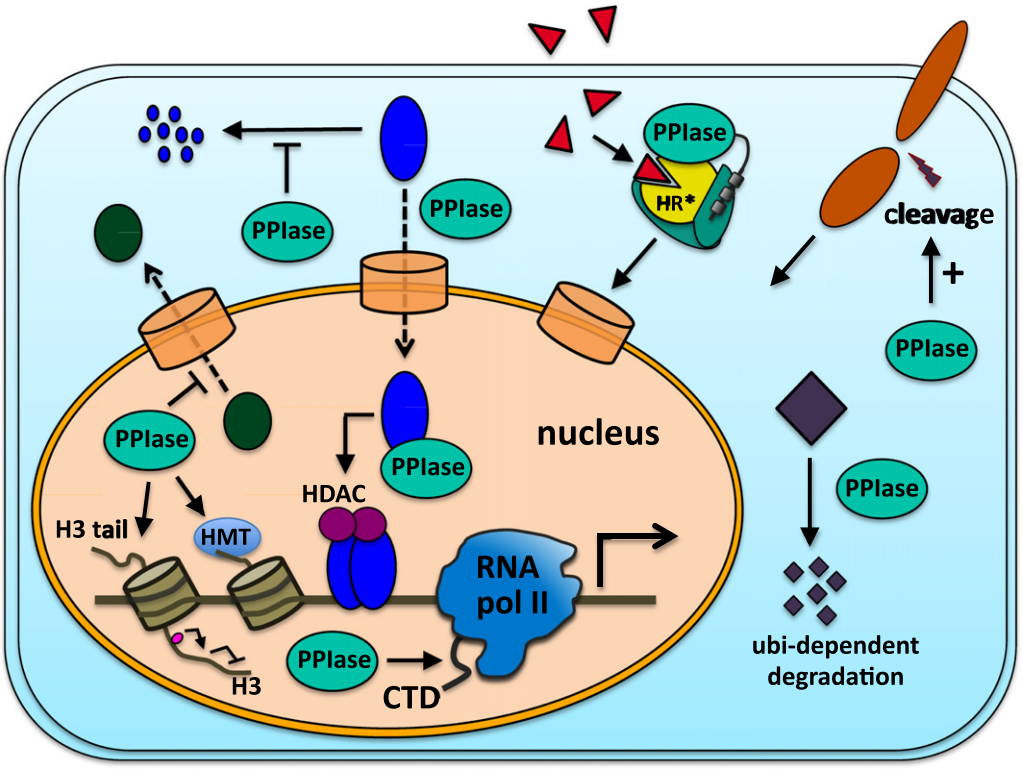

Fig. 7. Summary of the roles of prolyl isomerases in control of eukaryotic transcription. Examples of different mechanisms of regulation are shown starting with activation of membrane-bound transcription regulators by cleavage and release. The mechanisms include regulation of transcription factor stability, nuclear import and retention, ligand binding and activation of steroid hormone receptors (HR), co-repressor recruitment (HDACs), regulation of histone methyltransferases (HMT), regulation of histone tail modification by direct isomerization, and isomerization of the RNA polymerase CTD. Members of all three families (Fig. 1) participate in these mechanisms of regulation.

directed protein-interaction modules. Therefore, distinguishing catalytic from stoichiometric activity is necessary for understanding their function in a given pathway.

A major future challenge will be to carry out biochemical experiments using purified components to recapitulate *in vivo* observations, and to predict *in vivo* functional consequences of PPIase activities. For example, the effect of PPIases on nucleosome assembly, histone modification and *in vitro* transcription have all been carried out, but further work is needed in these and other areas. A related challenge will be to develop specific inhibitors, for example that can discriminate between different FKBPs or cyclophlins, or that inhibit parvulins specifically, without targeting unrelated enzymes. Developing FRET-type assays would also be useful for monitoring PPIase-induced conformation changes in proteins *in vivo*, and the effects of such changes on protein-protein interactions and the formation of protein complexes in cells. The fields of gene transcription and protein modification by prolyl isomerization have merged, and there is still much to learn.

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